REMARKS

FORMAL MATTERS

Claims 132-139, 141, and 143-153 are pending after entry of the amendments set forth herein. Claims 132-152 were examined and rejected. Claims 148-152 are pending.

Claims 140 and 142 are cancelled without prejudice.

Claim 132 has been amended for clarity and to incorporate claims 140 and 142. Claims 136, 138, 139, 141 and 143-146 have been amended for clarity. Support for new claim 153 is found in line 1 of page 6. No new matter is added.

Reconsideration of this application is respectfully requested.

INTERVIEW SUMMARY

The Applicants thank Examiner Li for the courtesy of a telephonic interview with the Applicants' representatives, James Keddie and Deno Dialynas, on April 8, 2008. All rejections and proposed arguments to address the same were discussed. No agreement was reached. Examiner Li is thanked for his advice.

Examiner Li is requested to kindly telephone James Keddie at (650) 833-7723 if the revised claims raise any new issues.

REQUEST FOR REJOINDER

Per MPEP § 821.04, the Applicants respectfully requests that, upon allowance of Claim 132 with respect to the elected species, the Examiner rejoin all claims which depend from or otherwise require all the limitations of Claim 132.

REJECTION OF CLAIMS UNDER 35 U.S.C. § 112, ¶1

Claims 132-139 and 145-147 are rejected as not meeting the written description and enablement requirements of 35 U.S.C. §112, first paragraph. This rejection is respectfully traversed.

As best understood by the Applicants, there are three aspects to this rejection. The first aspect relates to the overall description of the claimed method. The second and third aspects relate to the written description and enablement of the GPCRs recited in the claim.

It is believed that the first aspect of this rejection has been addressed by the provision of a revised set of claims. For example, the revised claims provide a nexus between the two determining steps (i.e., the compound identified in step c), which appears to be the root of the Examiner's issue.

With respect to the second and third aspects of this rejection relating to the genus of polypeptides recited in the claims, the Applicants initially note that the claims have been amended to recite "(c) identifying a compound as having an activity that stimulates said GPCR". As such, the amended claims require a *functional* GPCR having an amino acid sequence having at least 90% identity to SEQ ID NO:3, rather than any polypeptide having an amino acid sequence having at least 90% identity to SEQ ID NO:3. It is believed that this amendment – which requires that the GPCR used in the claimed methods be active – addresses the Examiner's concerns about the scope of the polypeptide recited in the rejected claims.

With further regard to the Examiner's concerns about the scope of the polypeptides recited in the claims, the Examiner is respectfully referred to the arguments presented on pages 8-10 of the Applicants' prior response, which appears to have been overlooked in formulating the current rejection. This section of the Applicants prior response discusses the structure/function relationship of GPCRs in detail and addresses both enablement and written description of the claims.

In addition, the Examiner is respectfully referred to pages 37-42 of the recently promulgated Written Description Training Materials (see www.uspto.gov/web/menu/written.pdf). While the Applicants understand that the fact pattern described in the Training Materials is different to the fact pattern of this case, the Applicants believe that the overall teachings of the Training Materials – which indicate that claims that recite a polypeptide having at least "85% amino acid sequence identity" to a disclosed polypeptide can meet the written description requirement even if there is little knowledge about the structure/functional relationship of the polypeptide – are directly

applicable to the instant case by analogy. Since there is an abundance of knowledge relating to the structure/function relationship of GPCRs, the Applicants submit that the instant claims, which recite "90% identity" language, are more than adequately described and enabled.

For the Examiner's convenience, the Applicants prior arguments are re-presented in quotes. Also, pages 37-42 of the recently Written Description Training Materials are enclosed herewith as Exhibit C. The Examiner is requested to reconsider these rejections in light of the Applicants prior arguments and the attached exhibits. All references cited in the following passage were cited in the Information Disclosure Statement of June 8, 2007.

The Applicants' prior arguments are provided in quotes below:

"It is the Applicant's understanding from a review of the Office Action that although two distinct and separate rejections are made under 35 U.S.C. §112, first paragraph, many of the issues that underpin both of the rejections are related, somewhat overlapping, and largely relate to the structural and functional characteristics of the polypeptides recited in the claims. As such, while the Applicants understand that the written description requirement of 35 U.S.C. §112 is separate and distinct from the enablement requirement of 35 U.S.C. §112[1] and that patentable subject matter needs to satisfy both requirements, the Applicant believes that both rejections can be addressed by a single discussion. In view of the above, the Examiner is requested to apply the following arguments to both of the rejections under 35 U.S.C. §112, first paragraph, as elaborated in this Office Action.

The rejected claims are directed to a screening method that employs a G protein-coupled receptor comprising an amino acid sequence having at least 90% identity to SEQ ID NO:3, a wild type human RUP41.

The basis for these rejections relates in large part to the claims encompassing variants of the human polypeptides that are explicitly disclosed in the specification. The questions are whether such molecules are adequately described in the specification, and whether one of skill in the art would make and use such molecules without undue experimentation.

---

[1] *In re Barker*, 559 F.2d 588, 194 USPQ 470 (CCPA 1977).

In response, the Examiner is respectfully directed to: a) page 2, line 33 to page 3, line 19 of the instant specification, where the structure/function relationship of GPCRs is described; b) page 4, lines 35 to page 5, line 4 of the instant specification, where two allelic variants of human RUP41, and a RUP41 from mouse are discussed; c) page 56, lines 10-12, where the specification provides guidance for making constitutively active mutants of RUP41; and d) the section starting on page 40, where a variety of methods for assaying GPCRs (which can be used to test variant proteins) are described in detail.

Further, as shown in Exhibit A, a search of NCBI's PubMed database reveals that there are well over 2900 journal articles, including 450 reviews, that have a publication date that precedes the priority date of the instant application (August 1, 2002) and contain the phrase "GPCR" OR "G protein-coupled receptor" in the abstract. Thus, at the priority date of the instant application, GPCR proteins were a subject of significant interest in the scientific community. The art in which the subject RUP41 protein belongs was therefore highly developed at the priority date of the instant application. For example, at the priority date of the instant application one of skill in the art would have knowledge of the atomic coordinates of at least one GPCR (see, e.g., reference A listed on Exhibit B). At the time of filing, the structure/function relationship of many GPCRs had been investigated (see, e.g., references B-H listed on Exhibit B), and several reviews on the structure/function relationship of GPCRs had been published (see, e.g., references I-O listed on Exhibit B)

In addition, at the time of filing, one of skill in the art would have been aware of several algorithms for predicting GPCR structure (see, e.g., references P and Q listed on Exhibit B), an algorithm for predicting important residues in GPCRs (see, e.g., reference R listed on Exhibit B), and reviews on the engineering of GPCRs by domain swapping (see, e.g., references S and T listed on Exhibit B).

Given the vast amount of available information on structure/function relationships in GPCR proteins in general, in combination with the structure/function information on RUP41 in the instant specification, the Applicant submits that one of skill in the art would be able to envision a large number of operable variants of RUP41, and be able to use those variants without undue experimentation.

The Applicant understands that the effect of amino acid and nucleotide substitutions cannot be predicted with absolute certainty. However, given the information in the instant specification and the deep general understanding of the structure and function of GPCR proteins, the Applicant submits that such molecules are more than adequately described and enabled.

The Applicant submits that these rejections have been adequately addressed. Withdrawal of these rejections is requested."

In addition to the above, the Applicants note that the claims have been amended to recite determining whether a compound *stimulates* the GPCR. Since such a step neither requires a constitutively active GPCR, nor a ligand for the GPCR, the Examiner's concerns outlined in the last paragraph of page 7 of the Office Action appear to be moot.

Finally, the Applicants provide herewith a number of publications that discuss the use of cultured cardiomyocytes as a model for heart disease. The abstracts of these publications with relevant sections underlined by the Applicants are submitted as Exhibits D to J, and the entire papers are submitted in an Information Disclosure Statement that accompanies this response. For example, Adams (Exhibit D) presents a review of the state of the art of cardiomyocytes and heart disease in 2001. In the abstract Adams states "Cardiomyocytes provide an ideal model system for understanding the basis for G-protein mediated hypertrophy and apoptosis.....This information may prove critical for designing interventions that prevent the pathophysiological consequences of heart failure". Statements underlined in the all of the other abstracts follow a similar vein. As such, it is clear that at the time of filing of the instant patent application cultured cardiomyocytes were an accepted, well-established model for heart disease, and that any concerns about whether cardioprotective agents can be discovered using cardiomyocytes would be unfounded. The Examiner is requested to consider the above in re-evaluating the amended claims.

The Applicants submit that this rejection has been adequately addressed. Withdrawal of this rejection is respectfully requested.

REJECTION OF CLAIMS UNDER 35 U.S.C. § 112, ¶2 (INDEFINITENESS)

Claims 132-147 are rejected under 35 U.S.C. § 112, second paragraph, for allegedly being indefinite.

It is believed that this rejection has been addressed by the provision of a revised set of claims. For example, the revised claims specify the relationship between claim 132 and of each of claims 136-139 and 144-146. Further, the claims now require a compound as having an activity that stimulates the GPCR, which the Applicants believe to be clear.

The Applicants submit that this rejection has been adequately addressed. Withdrawal of this rejection is respectfully requested.

CLAIM OBJECTIONS

Claim 144 is objected to for allegedly reciting non-elected subject matter.

The Applicants kindly request rejoinder of the non-elected species upon allowance of claim 132.

NEW CLAIMS

New claim 153 recites 95% identity language and is believed to be patentable for at least the reasons set forth above.

Conclusion

A timely Notice of Allowance is requested.

If the Examiner finds that a telephone conference would expedite the prosecution of this application, please telephone the undersigned at the number provided.

The Commissioner is hereby authorized to charge any underpayment of fees associated with this communication, including any necessary fees for extensions of time, or credit any overpayment to Deposit Account No. 50-0815, order number AREN-027.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: September 3, 2008

By: /James S. Keddie, Reg. No. 48,920/
James S. Keddie, Ph.D.
Registration No. 48,920

Enclosures: Exhibits A-J
IDS to cite references D to J

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F:\DOCUMENT\AREN\027\response to Office Action dated December 21, 2006.doc

10/523,100 Exhibit A

Items 1 - 20 of 2913 Page 1 of 146 Next

1: Medkova M, Preininger AM, Yu NJ, Hubbell WL, Hamm HE. Related Articles, Links

Conformational changes in the amino-terminal helix of the G protein alpha (i1) following dissociation from Gbetagamma subunit and activation.
Biochemistry. 2002 Aug 6;41(31):9962-72.
PMID: 12146960 [PubMed - indexed for MEDLINE]

2: Hengge UR, Ruzicka T, Tyring SK, Stuschke M, Roggendorf M, Schwartz RA, Seeber S. Related Articles, Links

Update on Kaposi's sarcoma and other HHV8 associated diseases. Part 2: pathogenesis, Castleman's disease, and pleural effusion lymphoma.
Lancet Infect Dis. 2002 Jun;2(6):344-52. Review.
PMID: 12144897 [PubMed - indexed for MEDLINE]

3: Whistler JL, Enquist J, Marley A, Fong J, Gladher F, Tsuruda P, Murray SR, Von Zastrow M. Related Articles, Links

Modulation of postendocytic sorting of G protein-coupled receptors.
Science. 2002 Jul 26;297(5581):615-20.
PMID: 12142540 [PubMed - indexed for MEDLINE]

4: Zhang N, Long Y, Devreotes PN. Related Articles, Links

Ege A, a novel C2 domain containing protein, is essential for GPCR-mediated gene expression in dictyostelium.
Dev Biol. 2002 Aug 1;248(1):1-12.
PMID: 12142016 [PubMed - indexed for MEDLINE]

5: Nickenig G. Related Articles, Links

Central role of the AT(1)-receptor in atherosclerosis.
J Hum Hypertens. 2002 Aug;16 Suppl 3:S26-33. Review.
PMID: 12140725 [PubMed - indexed for MEDLINE]

6: Poronnik P, Cummins MM, O'Mullane LM, Cook DI. Related Articles, Links

Use of adenoviruses to study isoform specificity of G-protein-receptor-coupled Ca2+ signaling in intact epithelial cells.
Cell Biochem Biophys. 2002;36(2-3):221-33. Review.
PMID: 12139408 [PubMed - indexed for MEDLINE]

7: Ehses JA, Pelech SL, Pederson RA, McIntosh CH. Related Articles, Links

Glucose-dependent insulinotropic polypeptide activates the Raf-Mek1/2-

EXHIBIT B

A. Palczewski et al, *Crystal structure of rhodopsin: A G protein-coupled receptor.* Science 2000 289:739-45.

B. Shin N et al, *Molecular modeling and site-specific mutagenesis of the histamine-binding site of the histamine H4 receptor.* Mol Pharmacol. 2002 62:38-47.

C. Chung DA et al, *Mutagenesis and peptide analysis of the DRY motif in the alpha2A adrenergic receptor: evidence for alternate mechanisms in G protein-coupled receptors.* Biochem Biophys Res Commun. 2002 293:1233-41.

D. Mouledous et al, *Functional inactivation of the nociceptin receptor by alanine substitution of glutamine 286 at the C terminus of transmembrane segment VI: evidence from a site-directed mutagenesis study of the ORL1 receptor transmembrane-binding domain.* Mol Pharmacol. 2000 57:495-502.

E. Krasnoperov et al, *Structural requirements for alpha-latrotoxin binding and alpha-latrotoxin-stimulated secretion. A study with calcium-independent receptor of alpha-latrotoxin (CIRL) deletion mutants*. J Biol Chem. 1999 274:3590-6.

F. Hurley et al, *Structure-function studies of the eighth hydrophobic domain of a serotonin receptor.* J Neurochem. 1999 72:413-21

G. Akal-Strader et al, *Residues in the first extracellular loop of a G protein-coupled receptor play a role in signal transduction*. J Biol Chem. 2002 277:30581-90.

H. Yang et al, *Molecular determinants of human melanocortin-4 receptor responsible for antagonist SHU9119 selective activity.* J Biol Chem. 2002 277:20328-35

I. Ulloa-Aguirre et al, *Structure-activity relationships of G protein-coupled receptors.* Arch Med Res. 1999 30:420-35 (Review)

J. Chollet et al, *Biophysical approaches to G protein-coupled receptors: structure, function and dynamics*. J Comput Aided Mol Des. 1999 13:209-19 (Review)

K. Gimpl et al, *The oxytocin receptor system: structure, function, and regulation.* Physiol Rev. 2001 81:629-83 (Review)

L. Bai et al, *Structure and function of the extracellular calcium-sensing receptor.* Int J Mol Med. 1999 4:115-25 (Review)

M. Olah et al, *The role of receptor structure in determining adenosine receptor activity*. Pharmacol Ther. 2000 85:55-75 (Review)

N. Missale et al, *Dopamine receptors: from structure to function*. Physiol Rev. 1998 78:189-225 (Review)

O. Sealfon et al, *Functional domains of the gonadotropin-releasing hormone receptor.* Cell Mol Neurobiol. 1995 15:25-42 (Review)

P. Filizola et al, *BUNDLE: a program for building the transmembrane domains of G-protein-coupled receptors*. J Comput Aided Mol Des. 1998 12:111-8.

Q. Orry et al, *Modeling and docking the endothelin G-protein-coupled receptor.* Biophys J. 2000 79:3083-94.

R. Califano *SPLASH: structural pattern localization analysis by sequential histograms.*

Bioinformatics. 2000 16:341-57.

S. Gouldson et al, *Domain swapping in G-protein coupled receptor dimers.* Protein Eng. 1998 11:1181-93.

T. Gouldson et al, *Dimerization and domain swapping in G-protein-coupled receptors: a computational study*. Neuropsychopharmacology. 2000 23:S60-77.

EXHIBIT C

Example 11: Percent Identity

11A: Art-Recognized Structure-Function Correlation Not Present

Specification:

The specification discloses a polynucleotide having the nucleic acid sequence of SEQ ID NO: 1, which encodes the polypeptide of SEQ ID NO: 2. The polypeptide of SEQ ID NO: 2 has the novel activity X, and does not share significant sequence identity with any known polypeptide or polypeptide family. The specification does not disclose any nucleic acid sequences that encode a polypeptide with novel activity X other than SEQ ID NO: 1.

Claims:

Claim 1. An isolated nucleic acid that encodes a polypeptide with at least 85% amino acid sequence identity to SEQ ID NO: 2.

Claim 2: An isolated nucleic acid that encodes a polypeptide with at least 85% amino acid sequence identity to SEQ ID NO: 2; wherein the polypeptide has activity X.

Analysis:

Claim 1

Claim 1 encompasses nucleic acids that encode the polypeptide of SEQ ID NO: 2, as well as those that encode any polypeptide having 85% structural identity to SEQ ID NO: 2. However, the specification discloses only a single species that encodes SEQ ID NO: 2; *i.e.*, SEQ ID NO: 1. There are no other drawings or structural formulas disclosed that encode either SEQ ID NO: 2 or a sequence with 85% identity to SEQ ID NO: 2.

The recitation of a polypeptide with at least 85% identity represents a partial structure, that is, at least 85% percent of the amino acids in the polypeptide will match those in SEQ ID NO: 2, and up to 15% of them may vary from those in SEQ ID NO: 2. However, there is no teaching regarding which 15% of the amino acids may vary from SEQ ID NO: 2. Consequently, there is also no information given about which nucleotides will vary from SEQ ID NO: 1 in the claimed genus of nucleic acids.

There is no functional limitation on the nucleic acids of claim 1 other than that they encode the polypeptide of SEQ ID NO: 2 or any polypeptide having 85% structural identity to SEQ ID NO: 2. The genetic code and its redundancies were known in the art before the application was filed.

The disclosure of SEQ ID NO: 2 combined with the pre-existing knowledge in the art regarding the genetic code and its redundancies would have put one in possession of the ge-

nus of nucleic acids that encode SEQ ID NO: 2. With the aid of a computer, one of skill in the art could have identified all of the nucleic acids that encode a polypeptide with at least 85% sequence identity with SEQ ID NO: 2. Thus, one of ordinary skill in the art would conclude that the applicant was in possession of the claimed genus at the time the application was filed.

Conclusion:

The specification satisfies the written description requirement of 35 U.S.C. 112, first paragraph, with respect to the scope of claim 1.

Claim 2

Claim 2 encompasses nucleic acids that encode the polypeptide of SEQ ID NO: 2, and nucleic acids that encode a polypeptide having 85% sequence identity to SEQ ID NO: 2 and have activity X. The specification discloses the reduction to practice of only a single species that encodes SEQ ID NO: 2 and has activity X; *i.e.*, SEQ ID NO: 1. There are no other drawings or structural formulas disclosed of a nucleic acid that encodes either SEQ ID NO: 2 or a polypeptide having 85% sequence identity to SEQ ID NO: 2 and activity X.

The claim includes a genus that can be analyzed at several levels sequentially for the purpose of focusing the issue.

First, the disclosure of SEQ ID NO: 2 combined with pre-existing knowledge in the art regarding the genetic code and its redundancies would have put one in possession of the genus of nucleic acids that encode SEQ ID NO: 2. With the aid of a computer, one of skill in the art could identify all of the nucleic acid sequences that encode a polypeptide with at least 85% sequence identity with SEQ ID NO: 2. However, there is no teaching regarding which 15% of the amino acids can vary from SEQ ID NO: 2 and still result in a protein that retains activity X. Further, there is no disclosed or art-recognized correlation between any structure other than SEQ ID NO: 2 and novel activity X.

An important consideration is that structure is not necessarily a reliable indicator of function. In this example, there is no disclosure relating similarity of structure to conservation of function. General knowledge in the art included the knowledge that some amino acid varia-

TECHNICAL NOTE

For information on amino acid substitution exchange groups and empirical similarities between amino acid residues, see a standard text such as Schulz et al., PRINCIPLES OF PROTEIN STRUCTURE, pp. 14-16, Springer-Verlag (New York 1979). There is a limit to how much substitution can be tolerated before the original tertiary structure is lost. Generally, tertiary structure conservation would be lost when the amino acid sequence varies by more than 50%. See, e.g., Cyrus Chothia and Arthur M. Lesk, "The relation between the divergence of sequence and structure in proteins," 5 THE EMBO JOURNAL 823-26 (1986).

tions are tolerated without losing a protein's tertiary structure. The results of amino acid substitutions have been studied so extensively that amino acids are grouped in so-called "exchange groups" of similar properties because substituting within the exchange group is expected to conserve the overall structure. For example, the expectation from replacing leucine with isoleucine would be that the protein would likely retain its tertiary structure. On the other hand, when non-exchange group members are substituted, e.g., proline for tryptophan, the expectation would be that the substitution would not likely conserve the protein's tertiary structure. Given what is known in the art about the likely outcome of substitutions on structure, those in the art would have likely expected the applicant to have been in possession of a genus of proteins having a tertiary structure similar to SEQ ID NO: 2 although the claim is not so limited.

However, conservation of structure is not necessarily a surrogate for conservation of function. In this case, there is no disclosed correlation between structure and function. The need for correlating information can vary. More specifically, those of skill in the art might require more or less correlating information depending on the kind of protein activity. If activity X is simply structural, e.g., a member of the collagen class, correlating information might not be a critical factor. However, if activity X is enzymatic, and there is no disclosure of the active site amino acid residues responsible for the catalytic activity, lack of that kind of correlating information may be a problem. Similarly, if activity X is as a ligand, and there is no disclosure of the domain(s) responsible for the ligand activity, the absence of information may be persuasive that those of skill in the art would not take the disclosure as generic.

Summarizing, there are no known or disclosed proteins having activity X other than SEQ ID NO: 2. As of the filing date, there was no known or disclosed correlation between a structure other than SEQ ID NO: 2 and activity X. While general knowledge in the art may have allowed one of skill in the art to identify other proteins expected to have the same or similar tertiary structure, in this example there is no general knowledge in the art about activity X to suggest that general similarity of structure confers the activity. Accordingly, one of skill in the art would not accept the disclosure of SEQ ID NO: 2 as representative of other proteins having activity X.

Conclusion:

The specification, taken with the pre-existing knowledge in the art of amino acid substitution and the genetic code, fails to satisfy the written description requirement of 35 U.S.C. 112, first paragraph, with respect to the scope of claim 2.

11B: ART-RECOGNIZED STRUCTURE-FUNCTION CORRELATION PRESENT

Specification:

The specification discloses a polynucleotide having the nucleic acid sequence of SEQ ID NO: 1, which encodes the polypeptide of SEQ ID NO: 2. The polypeptide of SEQ ID NO: 2

has a novel activity Y, and does not share significant sequence identity with any known polypeptide or polypeptide family. The specification does not disclose any nucleic acid sequences that encode a polypeptide with novel activity Y other than SEQ ID NO: 1. However, the specification discloses data from deletion studies that identify two domains as critical to activity Y, *i.e.*, a binding domain and a catalytic domain. The specification proposes that conservative mutations in these domains (e.g., one basic amino acid substituted for another basic amino acid) will still result in a protein having activity Y, whereas most non-conservative mutations in these domains will not result in a polypeptide having the recited activity. The specification also proposes that most mutations, conservative or non-conservative, outside the two domains will not affect activity Y to any great extent.

Claims:

Claim 1. An isolated nucleic acid that encodes a polypeptide with at least 85% amino acid sequence identity to SEQ ID NO: 2.

Claim 2. An isolated nucleic acid that encodes a polypeptide with at least 85% amino acid sequence identity to SEQ ID NO: 2; wherein the polypeptide has activity Y.

Analysis:

Claim 1

(This analysis proceeds the same as the analysis for claim 1 in Example 11A (Art-Recognized Structure-Function Correlation Not Present)

Claim 1 encompasses a vast genus of nucleic acids that encode the polypeptide of SEQ ID NO: 2, as well as those that encode any polypeptide having 85% structural identity to SEQ ID NO: 2.

The specification, however, discloses the reduction to practice of only a single species that encodes SEQ ID NO: 2, *i.e.*, SEQ ID NO: 1. There are no other drawings or structural formulas disclosed that encode either SEQ ID NO: 2, or a sequence with 85% identity to SEQ ID NO: 2.

Although the recitation of a polypeptide with at least 85% identity represents a partial structure -- in that 85% percent of the polypeptide is known, while 15% of the structure may vary -- there is no teaching regarding which 15% of the amino acids will vary from SEQ ID NO: 2. Consequently, there is also no information about which nucleotides will vary from SEQ ID NO: 1 in the claimed genus of nucleic acids.

There are no functional characteristics disclosed for the nucleic acids of claim 1 other than they encode the polypeptide of SEQ ID NO: 2 or any polypeptide having 85% structural identity to SEQ ID NO: 2. Further, the specification fails to disclose a method of making nucleic acids encoding polypeptides having 85% identity to SEQ ID NO: 2.

Nonetheless, the disclosure of SEQ ID NO: 2 combined with the knowledge in the art regarding the genetic code would put one in possession of the genus of nucleic acids that encode SEQ ID NO: 2. Further, with the aid of a computer, one could list all of the nucleic acid sequences that encode a polypeptide with at least 85% sequence identity with SEQ ID NO: 2. Additionally, the level of skill and knowledge in the art is such that one of ordinary skill would be able to use conventional sequencing and nucleic acid synthesis techniques to routinely generate and identify nucleic acids that encode the polypeptide of SEQ ID NO: 2, as well as those that encode any polypeptide having 85% structural identity to SEQ ID NO: 2. Thus, one of ordinary skill in the art conclude that the applicant would have been in possession of the claimed genus at the time of filing.

Conclusion:

The specification satisfies the written description requirement of 35 U.S.C. 112, first paragraph, with respect to the scope of claim 1.

Claim 2

Claim 2 encompasses a genus of nucleic acids that encode the polypeptide of SEQ ID NO: 2 and those that encode any polypeptide having 85% structural identity to SEQ ID NO: 2, wherein the polypeptide additionally has activity Y.

The specification, however, discloses the reduction to practice of only a single species that encodes SEQ ID NO: 2 and has activity Y, *i.e.*, SEQ ID NO: 1. There are no other drawings or structural formulas disclosed of a nucleic acid that encodes either (i) SEQ ID NO: 2 or (ii) a polypeptide with 85% sequence identity to SEQ ID NO: 2 wherein the polypeptide also has activity Y.

The disclosure of SEQ ID NO: 2 combined with the knowledge in the art regarding the genetic code would have put one in possession of the genus of nucleic acids that encode SEQ ID NO: 2. Further, with the aid of a computer, one could list all of the nucleic acid sequences that encode a polypeptide with at least 85% sequence identity to SEQ ID NO: 2. However, the specification fails to teach which of the nucleic acid sequences that encode a polypeptide with at least 85% sequence identity to SEQ ID NO: 2 encode a polypeptide having the required activity Y.

PRACTICE NOTE

This example deals only with the written description analysis of the claimed nucleic acids. Enablement issues that may be raised by the recited facts are not addressed here, but should be considered during examination. A separate rejection for nonenablement should be made when appropriate.

Nonetheless, the specification identifies two domains responsible for activity Y, *i.e.*, a binding domain and catalytic domain. The specification also predicts that conservative mutations in these domains will result in a protein having activity Y. Although all conservative amino acid substitutions in these domains will not nec-

essarily result in a protein having activity Y, those of ordinary skill in the art would expect that many of these conservative substitutions would result in a protein having the required activity. Further, amino acid substitutions outside of the two identified functional domains are unlikely to greatly affect activity Y. Thus, a correlation exists between the function of the claimed protein and the structure of the disclosed binding and catalytic domains. Consequently, there is information about which nucleic acids can vary from SEQ ID NO: 1 in the claimed genus of nucleic acids and still encode a polypeptide having activity Y. Based on the applicant's disclosure and the knowledge within the art, those of ordinary skill in the art would conclude that the applicant would have been in possession of the claimed genus of nucleic acids based on the disclosure of the single species of SEQ ID NO: 1.

Conclusion:

The specification satisfies the written description requirement of 35 U.S.C. 112, first paragraph, with respect to the scope of claim 2.

Exhibit D

1: Oncogene. 2001 Mar 26;20(13):1626-34.

G-proteins in growth and apoptosis: lessons from the heart.

Adams JW, Brown JH.

University of California, San Diego, Department of Pharmacology, 9500 Gilman Drive, 0636, La Jolla, CA 92093-0636, USA.

The acute contractile function of the heart is controlled by the effects of released nonepinephrine (NE) on cardiac adrenergic receptors. NE can also act in a more chronic fashion to induce cardiomyocyte growth, characterized by cell enlargement (hypertrophy), increased protein synthesis, alterations in gene expression and addition of sarcomeres. These responses enhance cardiomyocyte contractile function and thus allow the heart to compensate for increased stress. The hypertrophic effects of NE are mediated through Gq-coupled alpha(1)-adrenergic receptors and are mimicked by the actions of other neurohormones (endothelin, prostaglandin F(2alpha) angiotensin II) that also act on Gq-coupled receptors. Activation of phospholipase C by Gq is necessary for these responses, and protein kinase C and MAP kinases have also been implicated. Gq stimulated cardiac hypertrophy is also evident in transgenic mouse models. In contrast, stimulation of G(s)-coupled beta-adrenergic receptors or G(i)-coupled receptors do not directly effect cardiomyocyte hypertrophy. Apoptosis is also induced by G-protein-coupled receptor stimulation in cardiomyocytes. Sustained or excessive activation of either Gq- or Gs-signaling pathways results in apoptotic loss of cardiomyocytes both in vitro and in vivo. Apoptosis is associated with decreased ventricular function in the failing heart. Cardiomyocytes provide an ideal model system for understanding the basis for G-protein mediated hypertrophy and apoptosis, and the mechanisms responsible for the transition from compensatory to deleterious levels of signaling. This information may prove critical for designing interventions that prevent the pathophysiological consequences of heart failure.

PMID: 11313910 [PubMed - indexed for MEDLINE]

Related Links

Enhanced Galphaq signaling: a common pathway mediates cardiac hypertrophy and apoptotic heart failure. [Proc Natl Acad Sci U S A. 1998]

Gq-coupled receptor agonists mediate cardiac hypertrophy in vivo by angiotensin [Hypertension. 2002]

Dual actions of the Galpha(q) agonist Pasteurella multocida toxin to promote cardiomyocyte hypertrophy and enhance apoptosis susceptibility. [Circ Res. 2002]

Cardiac-specific overexpression of diacylglycerol kinase zeta prevents Gq protein-coupled receptor agonist-induced cardiac hypertrophy in [Circulation. 2006]

Mechanisms of impaired beta-adrenergic receptor signaling in G(alphaq)-mediated cardiac hypertrophy and ventricular dysfunction. [Mol Pharmacol. 2000]

» See all Related Articles...

Exhibit E

1: FEBS Lett. 2003 Feb 11;536(1-3):85-91.

H9c2 cardiac myoblasts undergo apoptosis in a model of ischemia consisting of serum deprivation and hypoxia: inhibition by PMA.

Bonavita F, Stefanelli C, Giordano E, Columbaro M, Facchini A, Bonafè F, Caldarera CM, Guarnieri C.

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Cardiac myocytes undergo apoptosis under condition of ischemia. Little is known, however, about the molecular pathways that mediate this response. We show that serum deprivation and hypoxia, components of ischemia in vivo, resulted in apoptosis of rat ventricular myoblast cells H9c2. Hypoxia alone did not induce significant apoptosis for at least 48 h, but largely increased the proapoptotic action of serum deprivation. H9c2 cells apoptosis is evidenced by an increase in terminal (TdT)-mediated dUTP nick end-labeling-positive nuclei and by activation of caspases 3, 6, 7 and 9, and loss of mitochondrial functions. In this model of simulated ischemia, represented by serum deprivation plus hypoxia, cardiomyoblasts apoptosis was associated with a p53-independent Bax accumulation and with a down-regulation of Bcl-xL, whereas the levels of cIAP-1, cIAP-2 and X-IAP proteins did not change. Phorbol-12-myristate-13-acetate significantly reduced the induction of apoptosis, inhibiting caspase 3 cleavage, Bax accumulation, Bcl-xL down-regulation as well as restoring cell viability.

PMID: 12586343 [PubMed - indexed for MEDLINE]

Exhibit F

1: Basic Res Cardiol. 1992;87 Suppl 2:19-31.

Links

Sympathetic modulation of the cardiac myocyte phenotype: studies with a cell-culture model of myocardial hypertrophy.

Long CS, **Kariya K**, **Karns L**, **Simpson PC**.

Cardiology Section, Veterans Administration Medical Center, San Francisco, California.

Myocardial hypertrophy is the common endpoint of many cardiovascular stimuli such as hypertension, myocardial infarction, valvular disease, and congestive failure. Catecholamines have long been implicated in the pathogenesis of myocardial hypertrophy, however, it is very difficult to sort out catecholamine mechanisms in vivo. We have developed a cell-culture model which excludes hemodynamic effects and allows the assignment of receptor specificity to catecholamine effects. Utilizing this system, we have shown that stimulation of the alpha 1 adrenergic receptor leads to the development of myocardial hypertrophy and results in the selective up-regulation of the fetal/neonatal mRNAs encoding skeletal alpha-actin and beta-MHC, a pattern similar to that seen with hypertrophy in-vivo. Utilizing a co-transfection assay, we have also obtained data that suggest that the beta-PKC isozyme is in a pathway regulating transcription of the beta-MHC isogene. Beta adrenergic stimulation of the cultured cardiac myocytes also results in a modest degree of hypertrophy, however, this effect may be dependent upon myocyte contractile activity and may involve, at least in part, the non-muscle cells present in the culture system.

PMID: 1338564 [PubMed - indexed for MEDLINE]

Related Links

Transcription of early developmental isogenes in cardiac myocyte hypertrophy. [J Mol Cell Cardiol. 1989]

Alpha 1-adrenergic receptor stimulation of sarcomeric actin isogene transcription in hypertrophy of cultured rat heart muscle cells. [J Clin Invest. 1989]

Sympathetic activity: modulator of myocardial hypertrophy? [J Cardiovasc Pharmacol. 1991]

Trophic factors for cardiac myocytes. [J Hypertens Suppl. 1990]

The cardiac beta-myosin heavy chain isogene is induced selectively in alpha 1-adrenergic receptor-stimulated hypertrophy of cultured rat heart myocytes. [J Clin Invest. 1990]

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Exhibit G

1: J Mol Med. 1997 Nov-Dec;75(11-12):901-20.

Various hypertrophic stimuli induce distinct phenotypes in cardiomyocytes.

Schaub MC, **Hefti MA**, **Harder BA**, **Eppenberger HM**.

Institute of Pharmacology, University of Zurich, Switzerland.

Cardiac hypertrophy is characterized by an increase in cell size in the absence of cell division and is accompanied by a number of qualitative and quantitative changes in gene expression. Most forms of hypertrophy in vivo are compensatory or adaptative responses to increased workload resulting from various physiological and/or pathological etiologies. Until severe pathological alterations become apparent, myocytes show no drastic morphological changes. On the level of gene expression, upregulation of the so-called fetal genes, i.e., beta-myosin heavy chain, alpha-skeletal and alpha-smooth muscle actin, and atrial natriuretic factor (ANF) may be observed concomitant with a downregulation of alpha-myosin heavy chain and the Ca pump of sarcoplasmic reticulum. The use of primary cell culture systems for cardiomyocytes as an in vitro model for the hypertrophic reaction has identified a number of different stimuli as mediators of cardiac myocyte hypertrophy. The molecular dissection of the different intracellular signaling pathways involved herein has uncovered a number of branching points to cytosolic and nuclear targets and has identified many interactions between these pathways. The individual administration of these hypertrophic stimuli, i.e., hormones, cytokines, growth factors, vasoactive peptides, and catecholamines, to cultured cardiomyocytes, reveals that each stimulus induces a distinct phenotype as characterized by gene expression pattern and cellular morphology. Surprisingly, triiodothyronine (T3) and basic fibroblast growth factor (bFGF) effect a similar cellular phenotype although they use completely different intracellular pathways. This phenotype is characterized by drastic inhibition of myofibrillar growth and by upregulation of alpha-smooth muscle actin. On the other hand, insulin-like growth factor (IGF) I, a factor

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Signaling pathways in cardiac myocyte hypertrophy. [J Mol Cell Cardiol. 1997]

Cardiotrophin-1 activates a distinct form of cardiac muscle cell hypertrophy. Assembly of sarcomeric units in series VIA gp130/leukemia inhibitory factor receptor-dependent pathways. [J Biol Chem. 1996]

Ras and rho are required for galphaq-induced hypertrophic gene expression in neonatal rat cardiomyocytes. [J Mol Cell Cardiol. 1998]

Renin-angiotensin system, hypertrophy and gene expression in cardiac myocytes. [J Mol Cell Cardiol. 1999]

Differential protein localization in sarcomeric and nonsarcomeric contractile structures of cultured cardiomyocytes. [J Struct Biol. 1998]

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promoting muscle cell differentiation, and bFGF, an inhibitor of differentiation, cause completely different cardiomyocyte phenotypes although both are known to signal via receptor tyrosine kinases and have been shown to activate the Ras-Raf-MEK-MAP kinase pathway. However, both IGF-I and bFGF depend on T3 to bring about their typical responses, i.e., T3 is permissive for the action of these two growth factors on the expression of alpha-smooth muscle actin and cell morphology. Most of the hypertrophic stimuli are balanced under normal circumstances in vivo. When this balance is disturbed, however, a pathological heart phenotype may become dominant. Thus the knowledge of signaling pathways and cellular responses triggered by hypertrophic stimuli may be essential for the implementation of therapeutic strategies in the treatment of cardiac hypertrophy.

PMID: 9428623 [PubMed - indexed for MEDLINE]

Exhibit H

1: Circ Res. 1999 Sep 3;85(5):403-14.

The mitochondrial apoptotic pathway is activated by serum and glucose deprivation in cardiac myocytes.

Bialik S, Cryns VL, Drincic A, Miyata S, Wollowick AL, Srinivasan A, Kitsis RN.

Departments of Medicine (Cardiology) and Cell Biology, Albert Einstein College of Medicine, Bronx, NY 10461, USA.

Many cell types undergo apoptosis under conditions of ischemia. Little is known, however, about the molecular pathways that mediate this response. A cellular and biochemical approach to elucidate such signaling pathways was undertaken in primary cultures of cardiac myocytes, a cell type that is especially sensitive to ischemia-induced apoptosis. Deprivation of serum and glucose, components of ischemia in vivo, resulted in myocyte apoptosis, as determined by nuclear fragmentation, internucleosomal cleavage of DNA, and processing of caspase substrates. These manifestations of apoptosis were blocked by zVAD-fmk, a peptide caspase inhibitor, indicating that caspase activity is necessary for the progression of apoptosis in this model. In contrast to control cells, apoptotic myocytes exhibited cytoplasmic accumulation of cytochrome c, indicating release from the mitochondria. Furthermore, both caspase-9 and caspase-3 were processed to their active forms in serum-/glucose-deprived myocytes. Caspase processing, but not cytochrome c release, was inhibited by zVAD-fmk, placing the latter event upstream of caspase activation. This evidence demonstrates that components of ischemia activate the mitochondrial death pathway in cardiac myocytes.

PMID: 10473670 [PubMed - indexed for MEDLINE]

Exhibit I

1: New Horiz. 1995 May;3(2):288-300.

Links

Molecular biology of myocardial hypertrophy and failure: gene expression and trophic signaling.

Parker TG.

Toronto Hospital, Department of Medicine, University of Toronto, ON, Canada.

Pressure-overload cardiac hypertrophy is associated with the re-expression of an ensemble of genes representative of embryonic myocardium, whose protein products modulate myocardial function. Regulation of cardiac-specific gene expression in end-stage myocardial disease in humans implies a pathophysiologic role for altered gene expression in the progression from compensatory hypertrophy to decompensated heart failure. The molecular signals that transduce load into a hypertrophic cardiac myocyte phenotype involve mechanical deformation and the local myocardial production of trophic factors, including angiotensin II, and transforming and fibroblast growth factors. Growth factors provoke a pattern of gene expression in cultured cardiac myocytes resembling that seen in pressure overload in vivo, in keeping with an autocrine or paracrine model of hypertrophy. Moreover, growth factor stimulation and pressure-overload hypertrophy share intracellular signaling pathways, including the activation of nuclear proteins encoded by cellular oncogenes. Elucidation of these signaling pathways may provide new therapeutic targets for the treatment of cardiac muscle disease that overcomes the limitations of currently available strategies.

PMID: 7583170 [PubMed - indexed for MEDLINE]

Exhibit J

1: Pharmacol Res. 2000 Jul;42(1):1-8. ELSEVIER Links

Isolated cardiac cells for electropharmacological studies.

Cerbai E, Sartiani L, De Paoli P, Mugelli A.

Department of Preclinical and Clinical Pharmacology, University of Firenze, Italy.

Single cardiac myocytes provide a model widely used to characterize the electrophysiological properties of drugs and to identify new therapeutic targets. This review focuses on isolation procedures to obtain single cardiac myocytes from several mammal species, including humans, and on patch-clamp technique as a useful method to investigate the molecular mechanism of druy actions. Copyright 2000 Academic Press.

PMID: 10860628 [PubMed - indexed for MEDLINE]

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Electropharmacological effects of antiarrhythmic drugs on atrial fibrillation termination. Part I: Molecular and ionic fundamentals of antiarrhythmic drug actions. [Ital Heart J. 2003]

Ionic remodeling in the heart: pathophysiological significance and new therapeutic opportunities for atrial fibrillation. [Circ Res. 2000]

Pharmacologic targets for atrial fibrillation. [Expert Opin Ther Targets. 2007]

Use of isolated adult myocytes to evaluate cardiotoxicity. II. Preparation and properties. [Toxicol Pathol. 1990]

An improved method for isolating cardiac myocytes useful for patch-clamp studies. [Am J Physiol. 1996]

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